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Characterization of *E. coli* Isolate and Assessment of its Antibiotic Susceptibility against Colistimethate Sodium

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ABSTRACT

Background*:* The increasing application of *Escherichia coli* as a model organism in microbiological quality control highlights importance of *E. coli* due to their wide range of applications. However, rising antimicrobial resistance in *E. coli* produced a serious concern with respect to public health globally which eventually generated the need of screening of pathogens for their sensitivity and resistance profile against different antibiotics.

Methods: The present study isolated a bacterial strain of *E. coli*, SA-01 from Ghaziabad, Uttar Pradhesh, India which was then characterized based on morphology, microscopy, biochemical testing in comparison with standard reference strains of *E. coli* (ATCC 8739, ATCC 10536 and MTCC 448). The identity of the isolated strain was further confirmed by MALDI-TOF analysis. The antibiotic susceptibility of the isolated strain of *E. coli* (SA-01) was then assessed in comparison with *E. coli* ATCC 10536 by cup plate method using different antibiotic doses of colistimethate sodium (1, 5, 10, 20, 30, 40, 50, 100 and 461 µg/mL).

Results: Based on phenotypic and biochemical characteristics, isolated bacterial strain, SA-01 was identified as *E. coli* and was further authenticated by MALDI-TOF analysis. In the antibiotic susceptibility testing, the indigenous strain of *E. coli* (SA-01) has shown zone of inhibition similar to *E. coli* ATCC 10536.

Conclusion: Similar zone of inhibition compared to *E. coli* ATCC 10536 in the antibiotic susceptibility testing, revealed sensitivity profile of the indigenous strain of *E. coli* (SA-01) to Colistimethate sodium suggesting its potential use in microbiological quality control methods.

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Introduction

Microbiological quality control methods are used in various industries such as the food, brewing, pharmaceutical, and biotechnology industries (1, 2). In the pharmaceutical industry, microbiological quality control (MQC) methods are very essential to assess the efficacy, quality and safety of the pharmaceutical products (3). Different types of tests used in microbiological quality control includes antimicrobial activity, sterility, bacterial endotoxin test (BET), environmental monitoring, and microbial limit test (MLT) (4). Some of the major bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., *Pseudomonas aeruginosa* etc. are extensively used in microbiological examination of pharmaceutical products (5).

Among them, *E. coli* is one of the important organisms majorly used in microbial assays of the pharmaceutical industry (6). *E. coli* is a Gram-negative, rod-shaped bacterium belongs to the *Enterobacteriaceae* family (7). *E. coli* is considered as a good model organism for bacterial evolution studies due to its adaptation to various growth environments and niches (8). The industrial manufacturing of recombinant proteins has greatly benefited by the use of *E. coli* (9). However, *E. coli* is considered as one of the most common pathogens causing variety of infections such as abdominal cramps, watery, mucoid, or bloody diarrhea, urinary tract infection syndromes, and meningitis (10). Besides, *E. coli* is considered as one of the most prevalent bacterial pathogens causing urinary tract infections (UTIs) in humans (11). *E. coli*, a fecal coliform provides more precise markers of fecal contamination which is now considered as the most effective indicator of feces-contaminated drinking water (12). *E. coli* is widely used as a standard strain in different microbiological assays for the assessment of various pharmaceutical products to check the sterility and efficacy (13). Standard strain of *E. coli*, ATCC 8739, has been used in quality control testing for antimicrobial hand washing formulations, media efficacy and bioresistance testing (14). Besides, other standard strains of *E. coli*, such as *E. coli* ATCC 10536 and ATCC 9637 have been reported to be commonly used in microbiological assay of antibiotics (cylinder plate method and turbidimetric method) to understand the susceptibility of pathogens (15, 16).

Antimicrobial resistance is a widespread public health issue that affects individuals throughout the world. Some of the harmful bacteria which were previously effectively treated with multiple antibiotics have developed resistance to most antibiotics currently. This antibiotic resistance has increased significantly in the recent period, and some of the bacteria have developed resistance to nearly all medicines (17). Especially, *E. coli* strains have developed resistance to popular antibiotics including β-lactams, carbapenemases, sulfonamides, and Fosfomycin etc. (18, 19). This rise of antibiotic resistance in *E. coli* presented a significant challenge to public health globally, eventually affecting the health care system (20). This increased resistance has developed an urgent need of susceptibility testing of pathogens against wide range of antimicrobial agents to determine the efficacy of antibiotics and understand the sensitivity profile of pathogens.

The current study was undertaken to isolate and identify *E. coli* strain from the sink sample, IPC, Ghaziabad followed by characterization and comparison with the standard reference strains of *E. coli*. Moreover, the isolated *E. coli* strain was analysed for its sensitivity and resistance patterns to Colistimethate sodium in comparison with the standard strain of *E. coli*, ATCC 10536.

Materials and Methods

Sampling and Isolation

Laboratory originated sewage sample was collected from the sink area of Microbiology Division, IPC, Ghaziabad. Sample from the sink area was collected using swab, which were processed on the same day.

The collected swab was spread plated on soybean casein digest agar (SCDA) (HiMedia) and then

plate was incubated at 35 ± 2 °C for 24 hours (21). After completion of incubation, plates were observed for colonies of bacteria. Based on colony morphology, we have picked single isolated colony resembling *E. coli* using wire loop and streak plated on fresh, sterile SCDA plates and incubated at 35 ± 2 °C for 24 hours. After completion of incubation, pure culture was subcultured on SCDA slants and stored in refrigerator at 4°C till the further use.

Microscopic study by Gram staining

Gram staining of an isolated culture of bacteria as well as 3 standard reference strains of *E. coli* [ATCC 8739, ATCC 10536, and MTCC 448 (equivalent to ATCC 9637)] from SCDA plates was performed to study the microscopic features (size, shape, color, and arrangement) by following standard method. Using a sterile wire loop, a pure culture was taken from SCDA plate and smear was prepared on clean glass slide, air dried and heat fixed. The smear was then stained with Gram's crystal violet stain for 1 minute and washed with tap water. Then smear was flooded with Gram's Iodine for 1 minute. Then, smear was washed with Gram's decolourizer until the blue dye no longer flows from the smear and again washed with tap water. Finally, counter stain (0.5% w/v safranin) was added on smear and kept for 20 seconds and then washed with water. After washing, slide was air dried and examined under Olympus microscope (23).

Motility test

Motility test was also performed for the isolated bacterium in comparison with 3 standard reference strains of *E. coli* (ATCC 8739, ATCC 10536, and MTCC 448). For this, a sterile inoculating needle was used to stab the motility medium (HiMedia) with bacterial suspension. The inoculated medium was incubated at 35 ± 2 °C for 24 hours. After the completion of incubation, tubes were observed for diffuse growth pattern in the medium (24).

Cultural characterization

The cultural characteristics of isolated pure culture and 3 standard reference strains of *E. coli* (ATCC 8739, ATCC 10536, and MTCC 448) were studied on different media such as SCDA, MacConkey and EMB agar (HiMedia). For this, a pure culture from slant was streaked on these media plates by four quadrant method and plates were incubated at 35 ± 2 °C for 24 hours (21). After incubation, colony characters (shape, size, surface, texture, edge, elevation, colour, opacity, etc.) were observed and noted.

Biochemical characterization

The isolated pure culture was subjected to biochemical characterization using different tests, such as indole, methyl red, Voges Proskauer, citrate, triple sugar iron, catalase, oxidase, nitrate, and phenol red dextrose agar with comparison to 3 standard reference strains of *E. coli* (ATCC 8739, ATCC 10536, and MTCC 448).

Indole test

A saline suspension of pure bacterial isolate was inoculated in test tube containing 3 mL DEV Tryptophan broth (HiMedia). Tubes were then incubated at 35 ± 2 °C for 24 hours. Upon incubation, 1-2 drops of KOVAC's reagent (HiMedia) was added to each tube, including the control, then gently mixed and the tubes were left to stand for 1-2 minutes. The tubes were then checked for the development of a cherry red ring and observations were noted (22).

Methyl red test

A suspension of pure bacterial isolate was inoculated in test tube containing buffered glucose broth (MR-VP medium) (HiMedia) and then tubes were incubated at 35 ± 2 °C for 24 hours. After incubation a drop of methyl red solution (HiMedia) was added and then tubes were checked for the development of brilliant red color (23).

Voges-Proskauer test

A suspension of pure bacterial isolate was inoculated in test tube containing buffered glucose broth (MR-VP medium) (HiMedia). Tubes were then incubated for 24 hours at 35 ± 2 °C. Then, 2-3 drops of Barritt reagent A (HiMedia) and Barritt reagent B (HiMedia) were added and tubes were then checked for the formation of pink colour as positive reaction and no colour change as negative reaction (24).

Citrate utilization test

A suspension of pure bacterial culture was streaked on the slants of Simmons citrate agar (HiMedia) and the slants were incubated at 35 ± 2 °C for 24 hours. After the incubation slants were checked for a change in colour from green to blue (22).

Phenol red dextrose agar test

A suspension of pure culture was streaked on the PRDA agar slants (HiMedia) and were then incubated at 35 ± 2 °C for 24 hours. After incubation, slants were observed for change in colour (yellow/red) (25).

Catalase test

The bacterial colony was mixed with a drop of 3% hydrogen peroxide (Fisher Scientific) on a sterile glass slide and slide was observed for the production of gas bubbles (24).

Oxidase test

A loopful of bacterial growth was rubbed using a sterile wire loop on an oxidase disc (HiMedia) in a sterile Petri plate. Then tubes were observed for the development of deep purple/blue color within 10 seconds (24).

Nitrate reduction test

A saline suspension of pure bacterial isolate was inoculated in test tube containing sterile nitrate broth (HiMedia) and tubes were incubated for 24 hours at 35 ± 2 °C. Upon incubation, 2-3 drops of each of the α-naphthylamine reagent (HiMedia) and sulphanilic acid (HiMedia) were added in the tubes. The tubes were then checked for the development of red color (24).

Triple sugar iron test

A suspension of pure bacterial isolate was streaked on the slants of TSI agar (HiMedia) and also stabbed till the bottom of the tube. The tubes were then incubated at 35 ± 2 °C for 24 hours. Then, the tubes were observed for change in color to yellow (acid production) or red (alkali production), or cracks in butt (gas production), or black color at the bottom of butt (H_2S production) (26).

Identification using MALDI-TOF

To confirm the identity of the isolated bacterial strain (SA-01), MALDI-TOF analysis using MALDI mass spectrometry TOF/TOF 5800 system (AB Sciex) was performed at NCCPF-PGIMER, Chandigarh, India.

Comparative antibiotic susceptibility testing

Isolated and identified indigenous strain of *E. coli* was subjected to antibiotic susceptibility testing in comparison with *E. coli* ATCC 10536. This antibiotic susceptibility test was performed by cup plate method using antibiotic assay Medium-H (HiMedia) by following regular procedure of Indian Pharmacopoeia (27). For this cylinder plate assay, initially a base layer was prepared by pouring around 21 mL molten, sterile media and allowed for solidification at room temperature for 30 minutes. Then, a 5 mL bacterial saline suspension was prepared from 24 hours old SCDA slants of test organism and reference strain ATCC 10536. Then transmittance of the bacterial suspension was

adjusted to 25% using UV-VIS Spectrophotometer (Perkin Elmer) for both test and reference strain of *E. coli*. A 100 µL of each suspension was transferred to the separate flask containing 100 mL of molten, cooled sterile Medium-H and mixed well. Then seed layer was prepared over the solidified base layer by pouring 4 mL of respective inoculated media and plates were allowed to solidify at room temperature for 30 minutes. Further, the wells were created by using 8 mm sterile borer. Different test concentrations of antibiotic Colistimethate sodium (1, 5, 10, 20, 30, 40, 50, 100 and 461 µg/mL) were prepared. A 100 µL of antibiotic solution of each concentration was inoculated in respective labelled well using micropipette and the plates were then incubated at 35 ± 2 °C for 24 hours. After completion of incubation, the zone of inhibitions (in mm) of each set of plates were measured using Vernier calliper (Mitutoyo) and observations were noted down.

Results

Sampling and Isolation

A swab of sink sample was collected from laboratory, IPC Ghaziabad, India and labelled as SA (Figure 1).

We have successfully isolated a bacterial strain resembling *E. coli* from the collected sample and labelled it as SA-01 (Figure 2).

Microscopic study by Gram staining

Microscopic observations of the isolated strain, SA-01 revealed Gram negative, short rods arranged in pairs or singly similar to standard reference strains of *E. coli* (Figure 3).

Motility test

The isolated bacterium SA-01 was found to be motile that shows diffuse, hazy growth through motility media similar to 3 standard reference strains of *E. coli* (ATCC 8739, MTCC 448, and ATCC 10536) (Figure 4).

(a) (b)

Figure 1. (a) Sink area, (b) Swab of sink sample from IPC laboratory.

Figure 2. Isolated bacterium (SA-01) on SCDA plate.

Cultural characterization

The organism shows circular, smooth, shiny, mucoid greyish white to yellowish colour colonies on SCDA (primary media), pink non-mucoid colonies on MacConkey agar (secondary media) and colonies with green metallic sheen on EMB agar (secondary media) similar to 3 standard reference strains of *E. coli* (ATCC 8739, MTCC 448, and ATCC 10536) (Figure 5).

Biochemical characterization

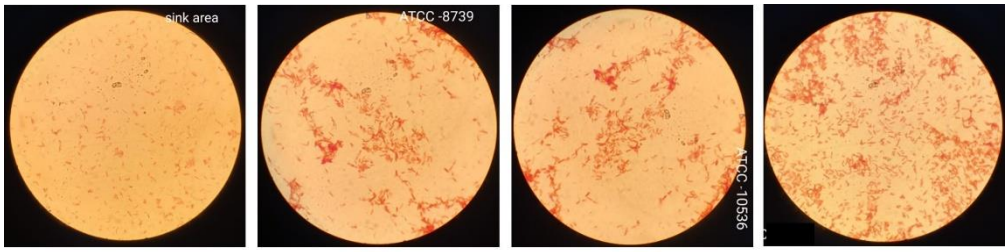
The isolated bacterial strain SA-01 has shown positive results for Catalase, Indole, Methyl Red, Phenol red dextrose agar, Nitrate reduction tests and negative for Oxidase, Voges-Proskauer, and Citrate, which were similar to all three standard

Table 1. Biochemical testing of isolated culture along with standard reference strains.

Sr. No.	Biochemical test	*E. coli* ATCC 8739	*E. coli* MTCC 448	*E. coli* ATCC 10536	SA-01
1.	Catalase	(+) ve	(+) ve	(+) ve	(+) ve
2.	Oxidase	(-) ve	(-) ve	(-) ve	(-) ve
3.	Indole	(+) ve	(+) ve	(+) ve	(+) ve
4.	Nitrate	(+) ve	(+) ve	(+) ve	(+) ve
5.	Methyl Red	(+) ve	(+) ve	(+) ve	(+) ve
6.	Voges-Proskauer	(-) ve	(-) ve	(-) ve	(-) ve
7.	Citrate	(-) ve	(-) ve	(-) ve	(-) ve
8.	PRDA	(+) ve	(+) ve	(+) ve	(+) ve
9.	TSI	A/A, Gas	A/A, Gas	A/A, Gas	K/A Gas
10.	Motility	(+) ve	(+) ve	(+) ve	(+) ve

Table 2. Antibiotic Susceptibility against Colistimethate sodium.

Test organism	Diameter of zone of inhibition (in mm) against Colistimethate sodium								
	01 µg/mL	05 µg/ mL	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL	461 µg/mL
***E. coli* ATCC 10536**	No zone	Not measurable	11.8	12.9	13.3	14	14.8	16.2	17.1
SA-01	No zone	Not measurable	10.1	11.5	12.4	13	13.8	14.8	16.9



SA-01 | ***E. coli* ATCC 8739** | ***E. coli* ATCC 10536** | ***E. coli* MTCC 448**

Figure 3. Gram staining of isolated culture and standard reference strains.

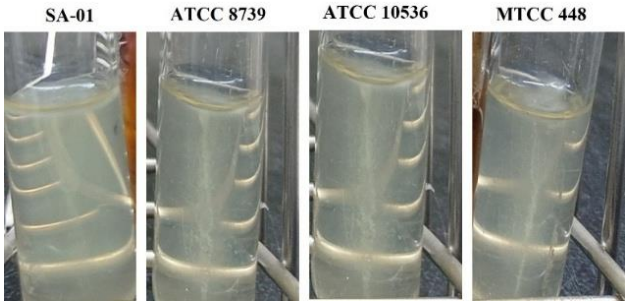


Figure 4. Motility test of isolated culture and standard reference strains.

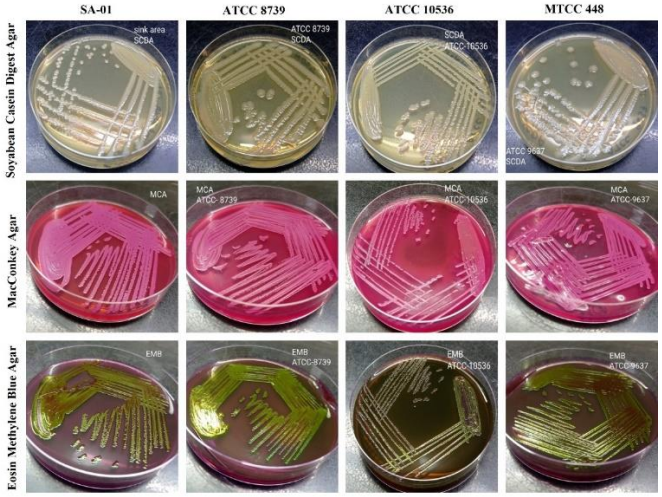


Figure 5. Culture characteristics of isolated culture and standard reference strains.

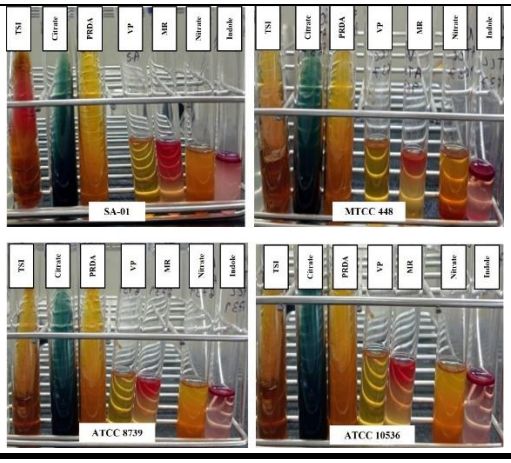


Figure 6. Biochemical characteristics of isolated culture and standard reference strains.

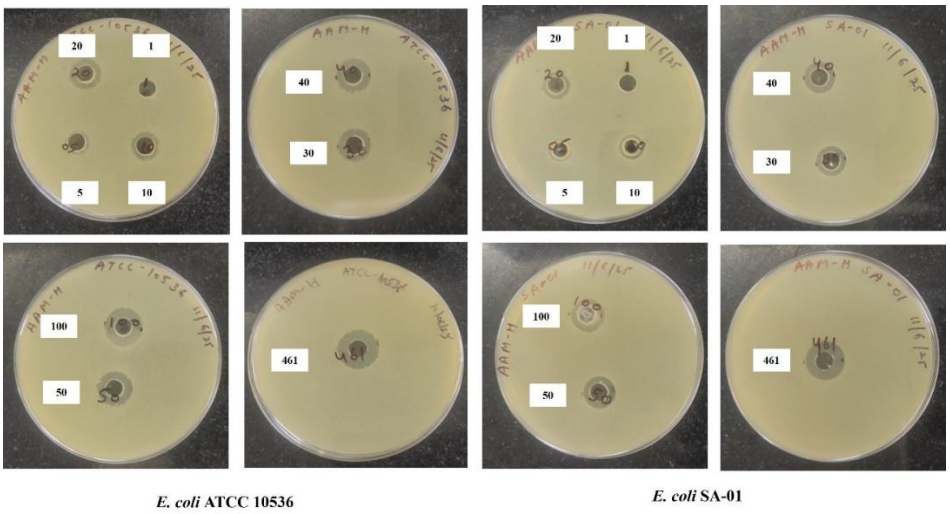


Figure 7. Antibiotic susceptibilities of isolated culture and standard reference strains.

reference strains of *E. coli* (ATCC 8739, MTCC 448, and ATCC 10536) except for TSI (Table 1, Figure 6).

Identification using MALDI-TOF

The MALDI-TOF analysis confirmed the identity of the isolated bacterial strain (SA-01) as *E. coli*.

Comparative antibiotic susceptibility testing

In antibiotic susceptibility test, the isolated strain SA-01 has shown positive results reporting prominent zone of inhibitions at 10, 20, 30, 40, 50, 100 and 461 µg/mL of Colistimethate sodium. However, it has shown no zone of inhibitions at lower concentrations (01 and 05 µg/mL) of Colistimethate sodium. This displayed that isolated strain is sensitive to Colistimethate sodium at 10 µg/mL and above similar to standard reference strain ATCC 10536 (Table 2, Figure 7).

Discussion

The rising and burning problem of antibiotic resistance in Gram-negative bacteria represents the major clinical issue globally which highlights the need for novel antibiotics (28). Besides, the exploration of novel indigenous susceptible strains is crucial for determining the effectiveness of potential drug candidates in the pharmaceutical industry (29).

The current research study reported a novel indigenous strain SA-01 from sewage sample of sink area. The microscopic observations showed that the isolated strain (SA-01) found to be microscopically similar to standard reference strains of *E. coli* (ATCC 8736, MTCC 448, and ATCC 10536). The present isolate was found to be Gram-negative, rod-shaped present in pairs or singly which coincides with microscopy of the standard reference strains. Similarly, the SA-01 has shown similar results in biochemical tests in comparison with all 03 standard reference strains of *E. coli*, except for TSI test. The SA-01 has shown circular, smooth, shiny and mucoid greyish white or yellowish coloured colonies on SCDA, pink non-mucoid colonies on MacConkey agar and colonies with green metallic sheen on EMB agar which matches with colony characteristics of standard reference strains. The similar microscopic, cultural and biochemical characteristic showed that the present isolate could be a probable *E. coli* strain. Moreover, the

MALDI-TOF analysis confirmed identity of the SA-01 as *E. coli*.

Colistimethate sodium is a broad-spectrum antibiotic belonging to Polymyxin class specifically active against Gram-negative bacteria (30). Considering its broad-spectrum activity, it was used for the antibiotic susceptibility testing of the present isolate in comparison with standard reference strain (*E. coli* ATCC 10536). Different concentrations of Colistimethate sodium were assessed to check the susceptibility of both the bacterial strains. The SA-01 has shown 16.9, 14.8, 13.8, 13, 12.4, 11.5 and 10.1 mm zone of inhibitions respectively at 461 µg/mL, 100 µg/mL, 50 µg/mL, 40 µg/mL, 30 µg/mL, 20 µg/mL, 10 µg/mL of Colistimethate sodium. Whereas standard reference strain has shown 17.1, 16.2, 14.8, 14, 13.3, 12.9 and 11.8 mm zone of inhibitions respectively at 461 µg/mL, 100 µg/mL, 50 µg/mL, 40 µg/mL, 30 µg/mL, 20 µg/mL, 10 µg/mL Colistimethate sodium. This clearly showed that, the present strain was found to be susceptible to tested antibiotic Colistimethate sodium showing prominent zone of inhibitions at higher concentrations of Colistimethate sodium (10 & >10 µg/mL). However, at lower concentrations of Colistimethate sodium (1 & 5 µg/mL), the strain SA-01 showed no zone of inhibition similar to standard reference strain. Overall, the antibiotic susceptibility results revealed that the present indigenous strain, *E. coli* SA-01 is sensitive to Colistimethate sodium similar to standard reference strain depicting its promising use for the assessment of efficacy of potential drugs.

Conclusion

The present study reported an indigenous strain of *E. coli* SA-01, showing phenotypic, microscopic and biochemical characteristics similar to standard reference strains. Additionally, the comparative screening of this indigenous isolate (SA-01) for its antibiotic susceptibility against Colistimethate sodium along with standard reference strain, *E. coli* ATCC 10536, revealed the sensitivity profile of the present isolate confirming its sensitivity to Colistimethate Sodium.

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Ethics approval and consent to participate

Not applicable.

Conflict of interest

Komal Parashar, Anil Kumar Teotia*, Prasad Thota, Ajay Chandrakant Lagashetti, Manoj Kumar Pandey, Ajayendra Kumar Keshari, Meenakshi Dahiya, and V. Kalaiselven declare that they have no conflict of interest.

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